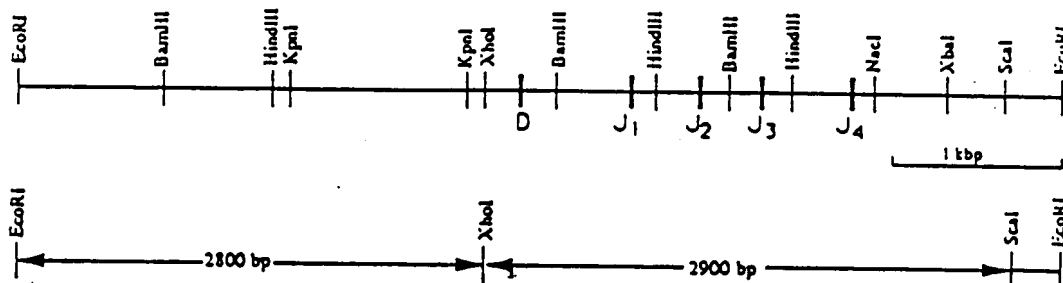


Mouse Heavy Chain J Genes Inactivation Vector

(A) Targeted mouse heavy chain J genes



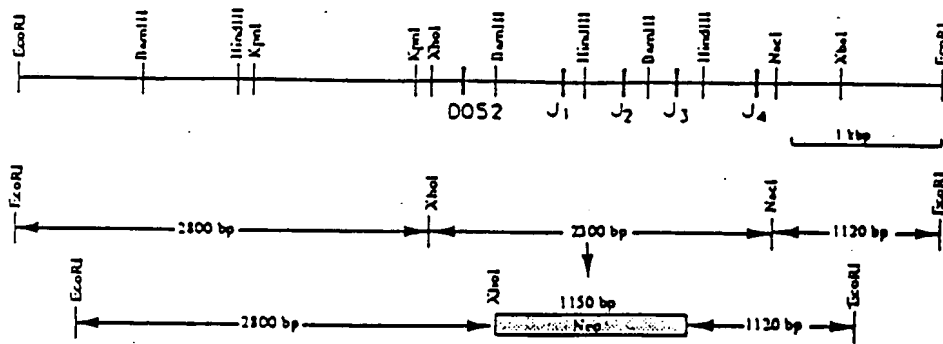
(B) Inactivation vector mDAI.Neo



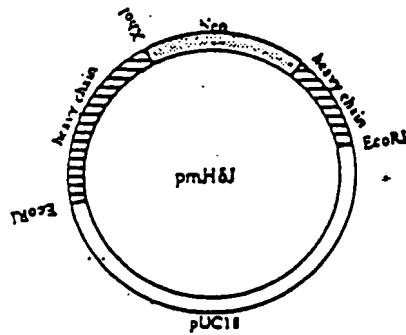
Figure 1

EI124 99007205

(A) Targeted mouse heavy chain *J* genes

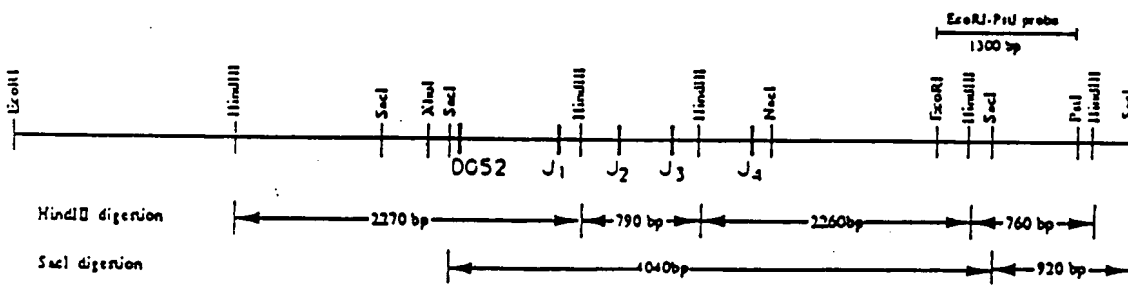


(B) Inactivation vector pmH8J



(C) Southern analysis of pmH8J-targeted ES colonies

Wild type ES cell genome



Targeted ES cell genome

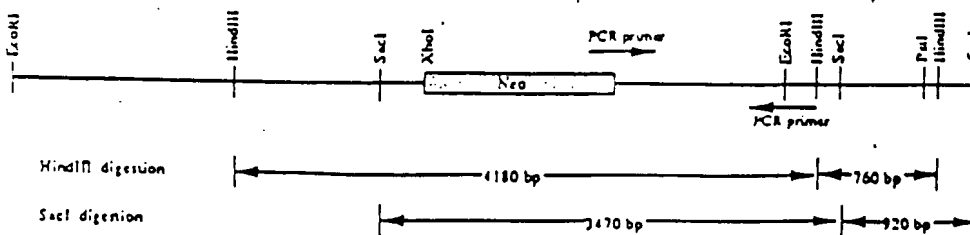
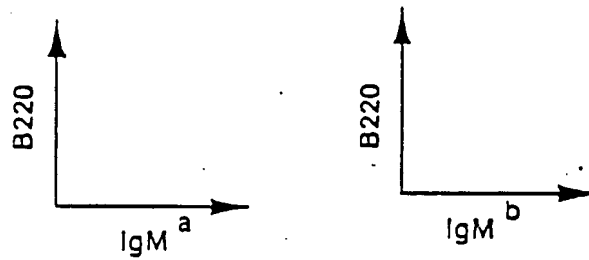
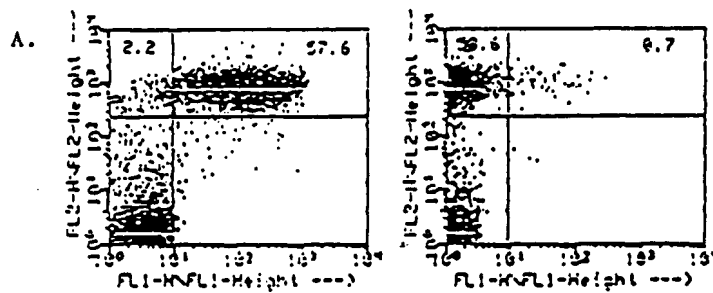


Figure 2

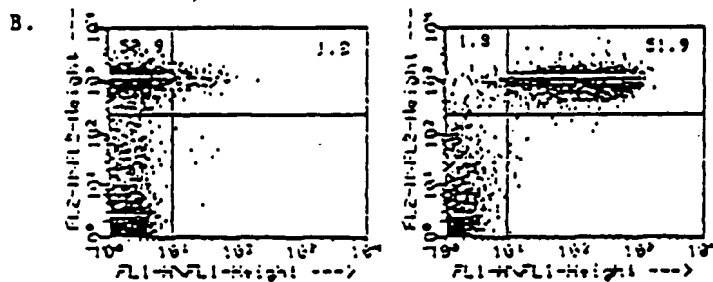
J_H deletion blocks cell surface IgM expression



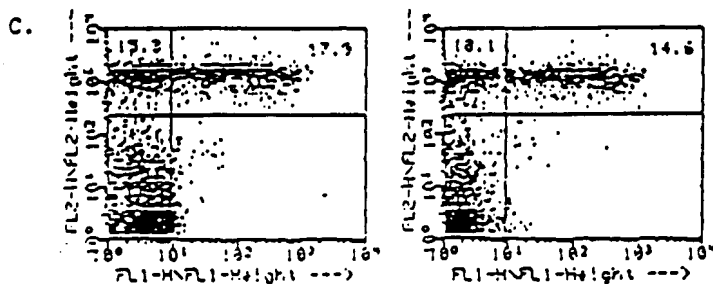
a allotype



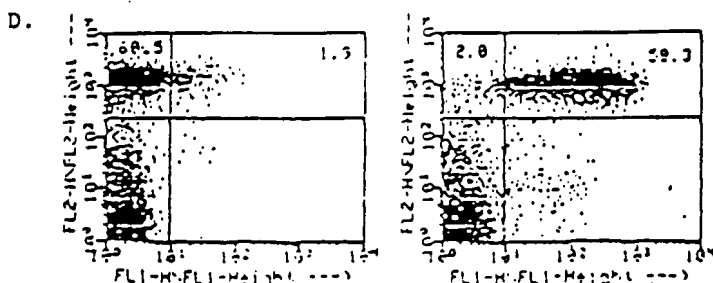
b allotype



a/b F1



ΔJ_H / b F1



Staining of peripheral blood lymphocytes with fluorescent anti-a allotype (A, D), anti-b allotype (B, E) or anti-B220 (C, F). (A, B, C) JH-deletion homozygous mutant mouse 244-3-2/F2-7, (D) A allotype control mouse, (E) B allotype control mouse. The number in each panel indicates the percentage of cells stained with the specific antibody.

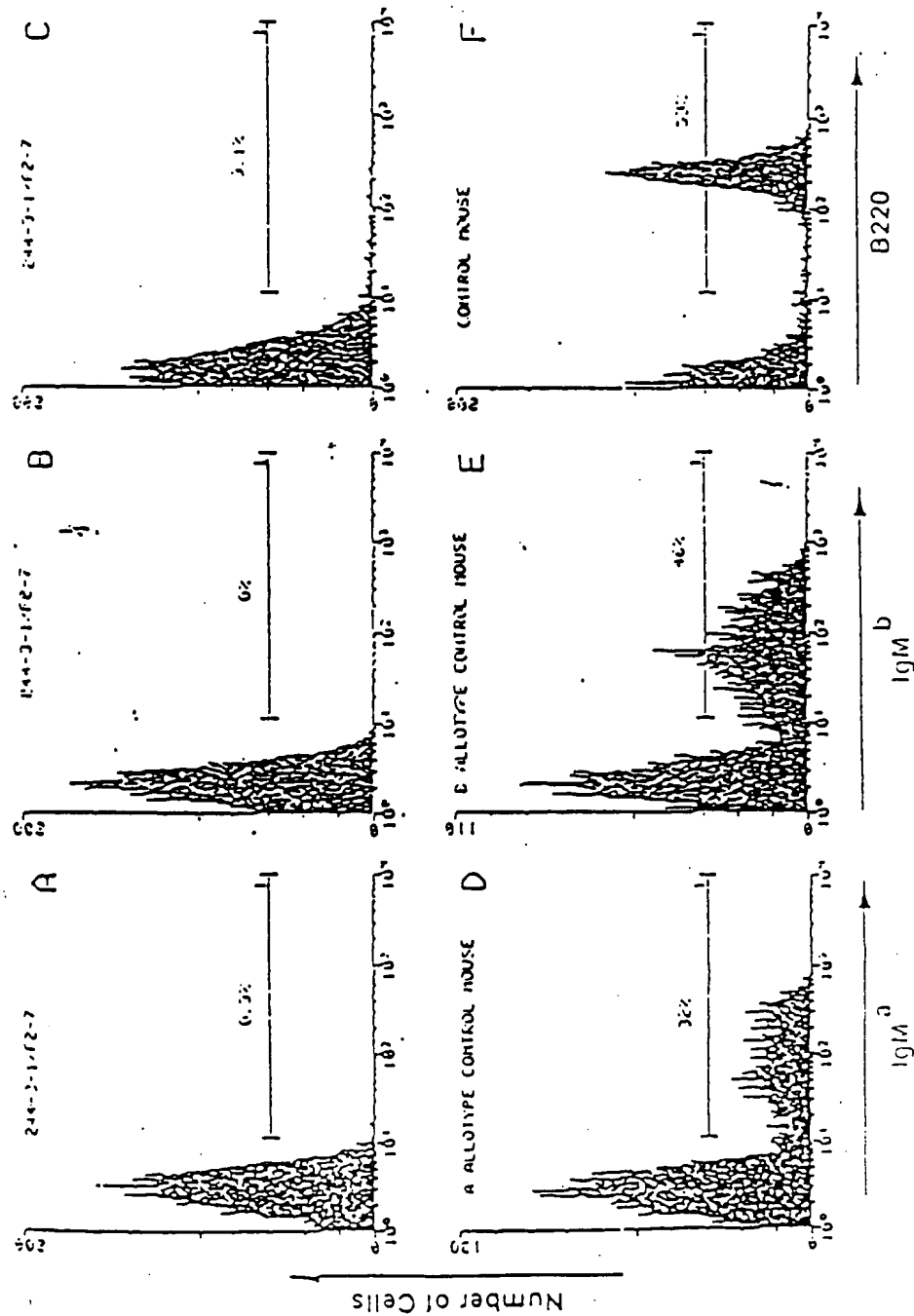


Figure 4

INACTIVATION OF KAPPA CONSTANT REGION

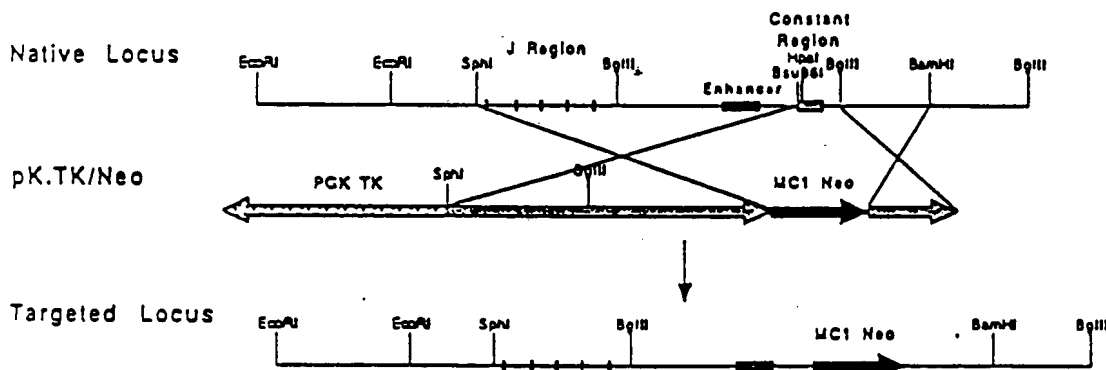
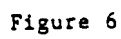
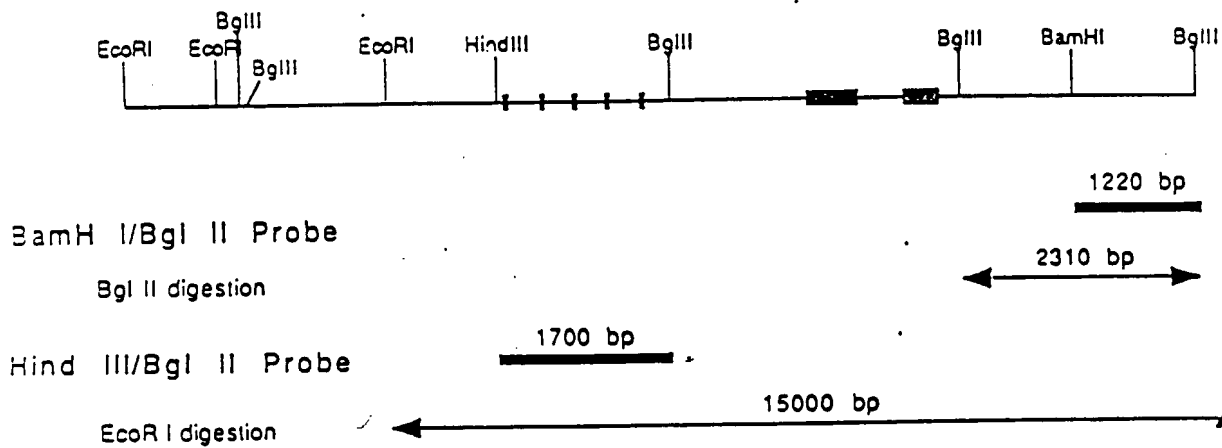


Figure 5



SOUTHERN ANALYSIS OF LIGHT CHAIN C_K-TARGETED E14-1 CELLS

NATIVE ES CELL LOCUS



TARGETED ES CELL LOCUS

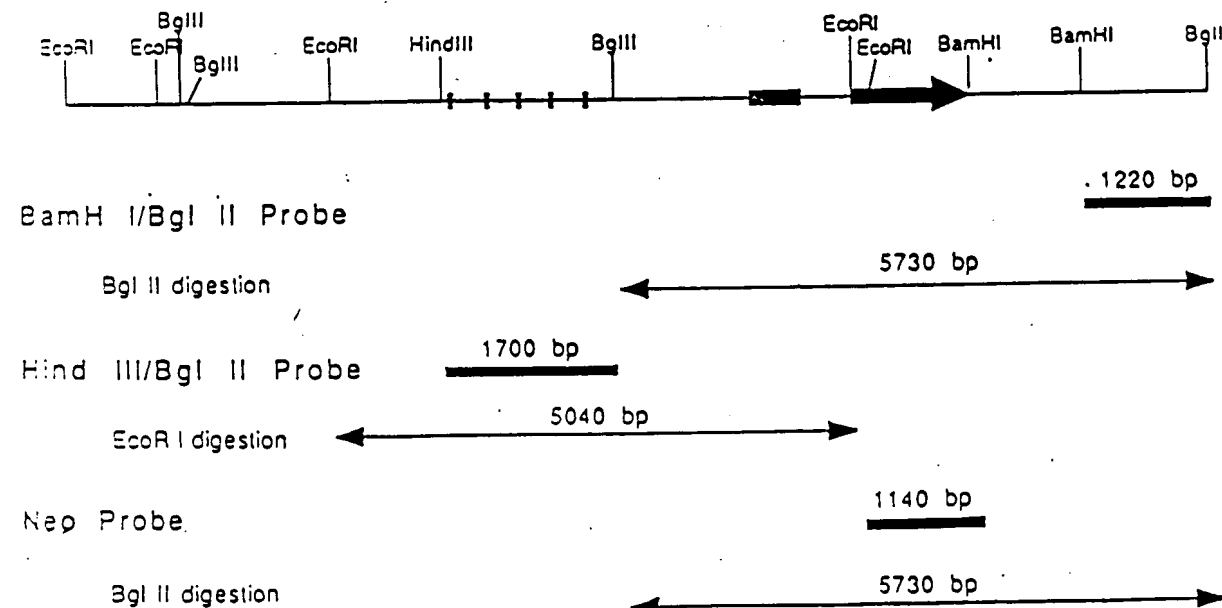
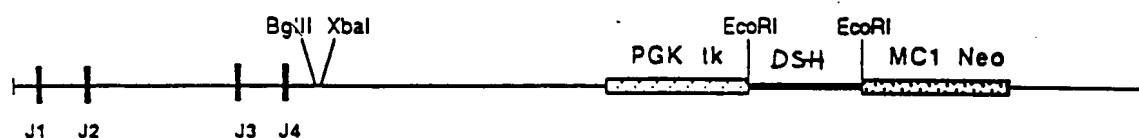


Figure 7

KAPPA J/CONSTANT REGION INACTIVATION

J REGION KNOCKOUT VECTOR



TARGETING SCHEME

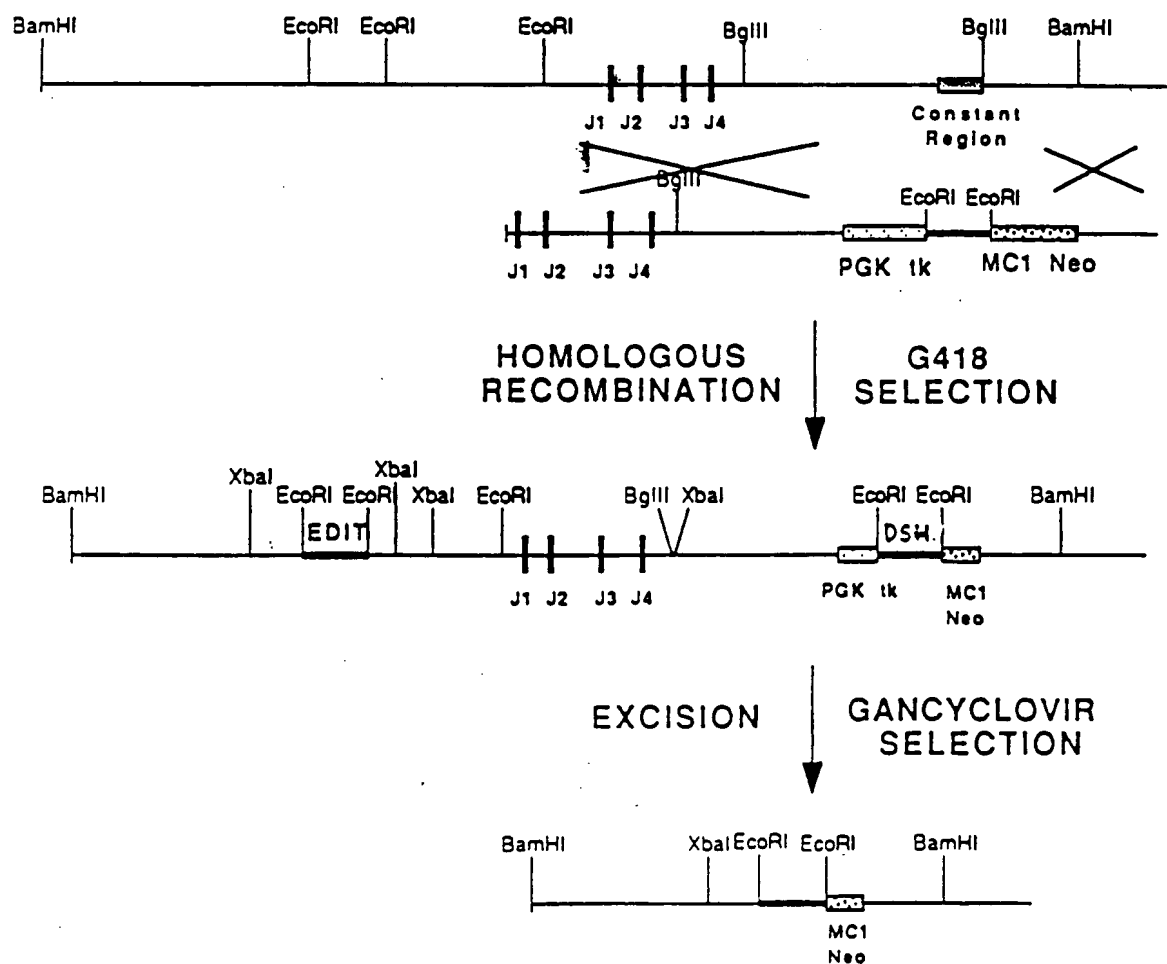


Figure 8

CONSTRUCTION OF KAPPA J/CONSTANT REGION DELETION VECTORS

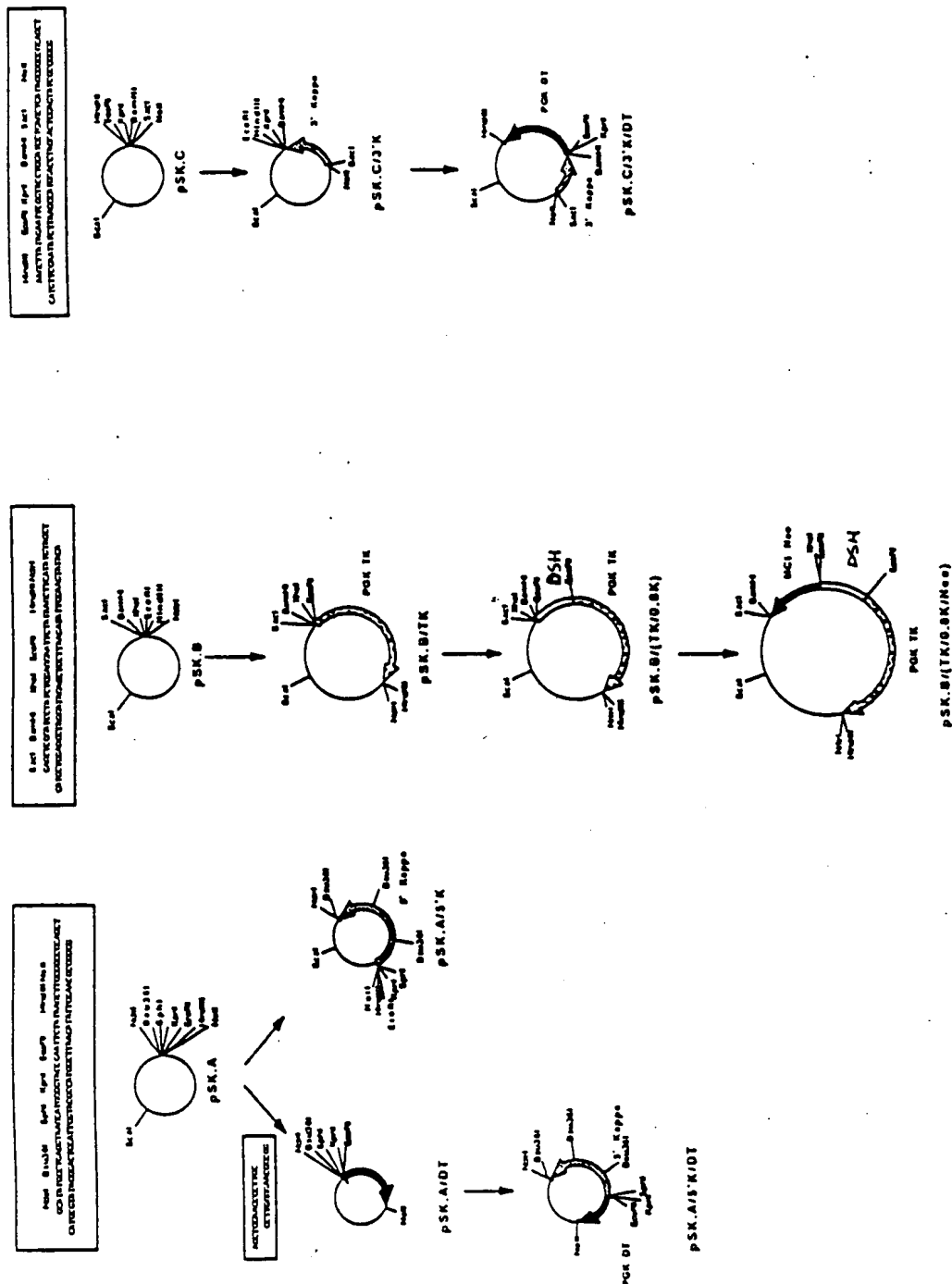


Figure 9

KAPPA J/CONSTANT REGION DELETION VECTORS

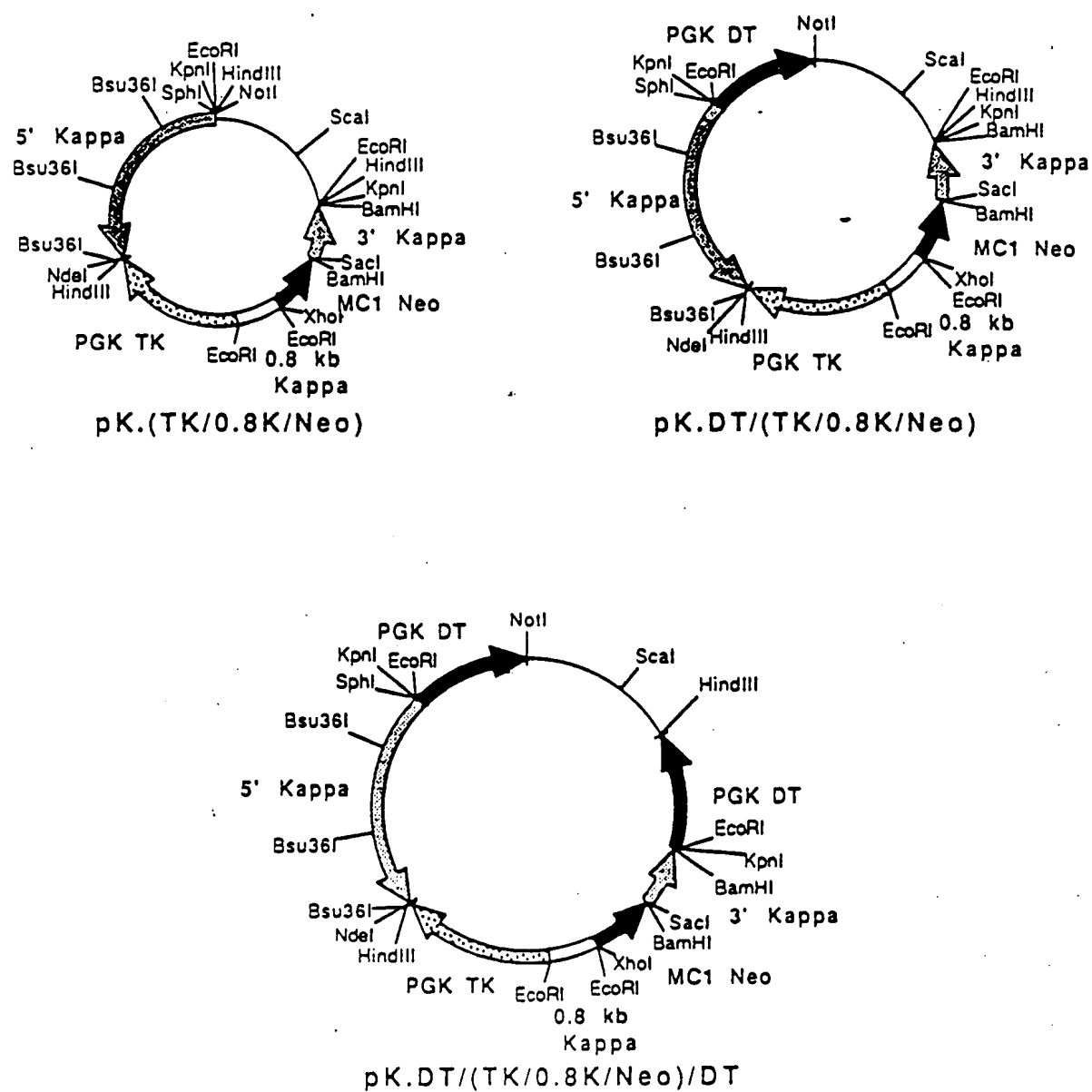
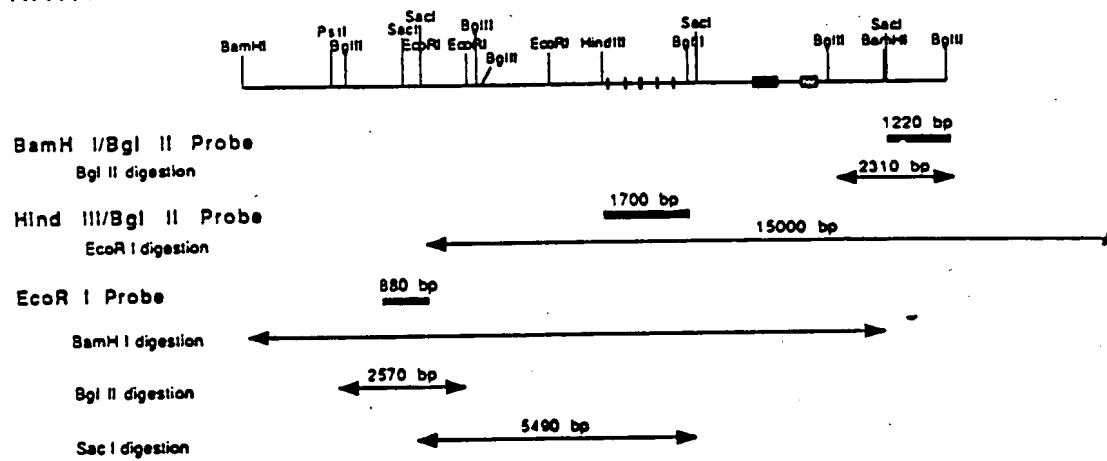


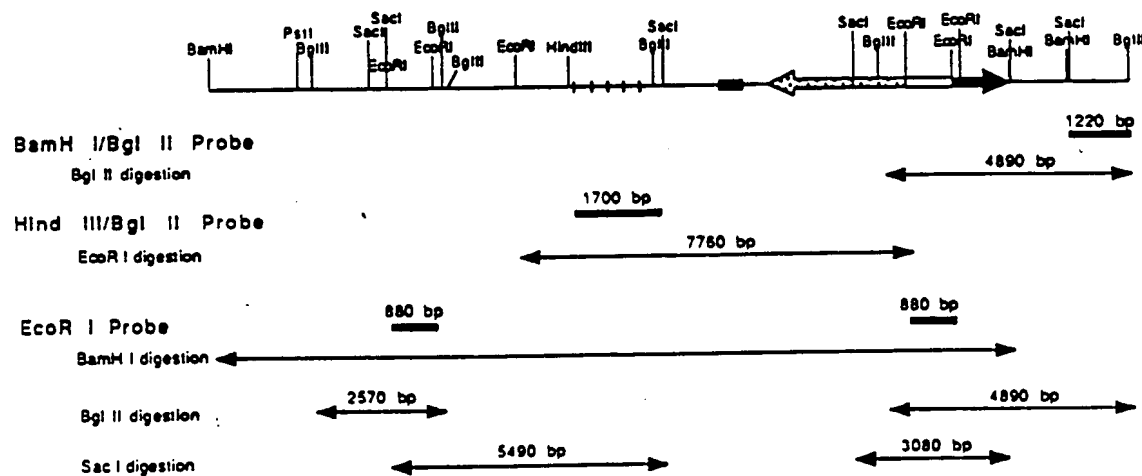
Figure 10

SOUTHERN ANALYSIS OF LIGHT CHAIN J κ /C κ -DELETED E14-1 CELLS

NATIVE ES CELL LOCUS



C κ -TARGETED ES CELL LOCUS



J κ C κ -DELETED ES CELL LOCUS

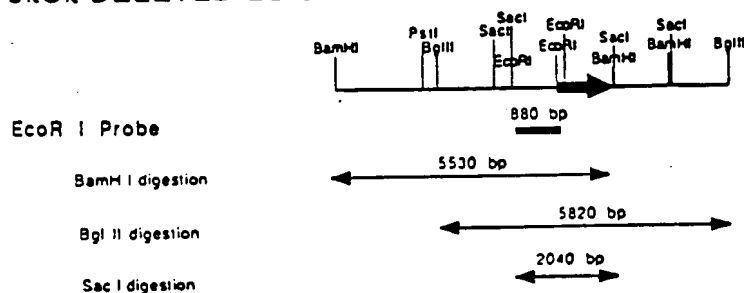


Figure 11

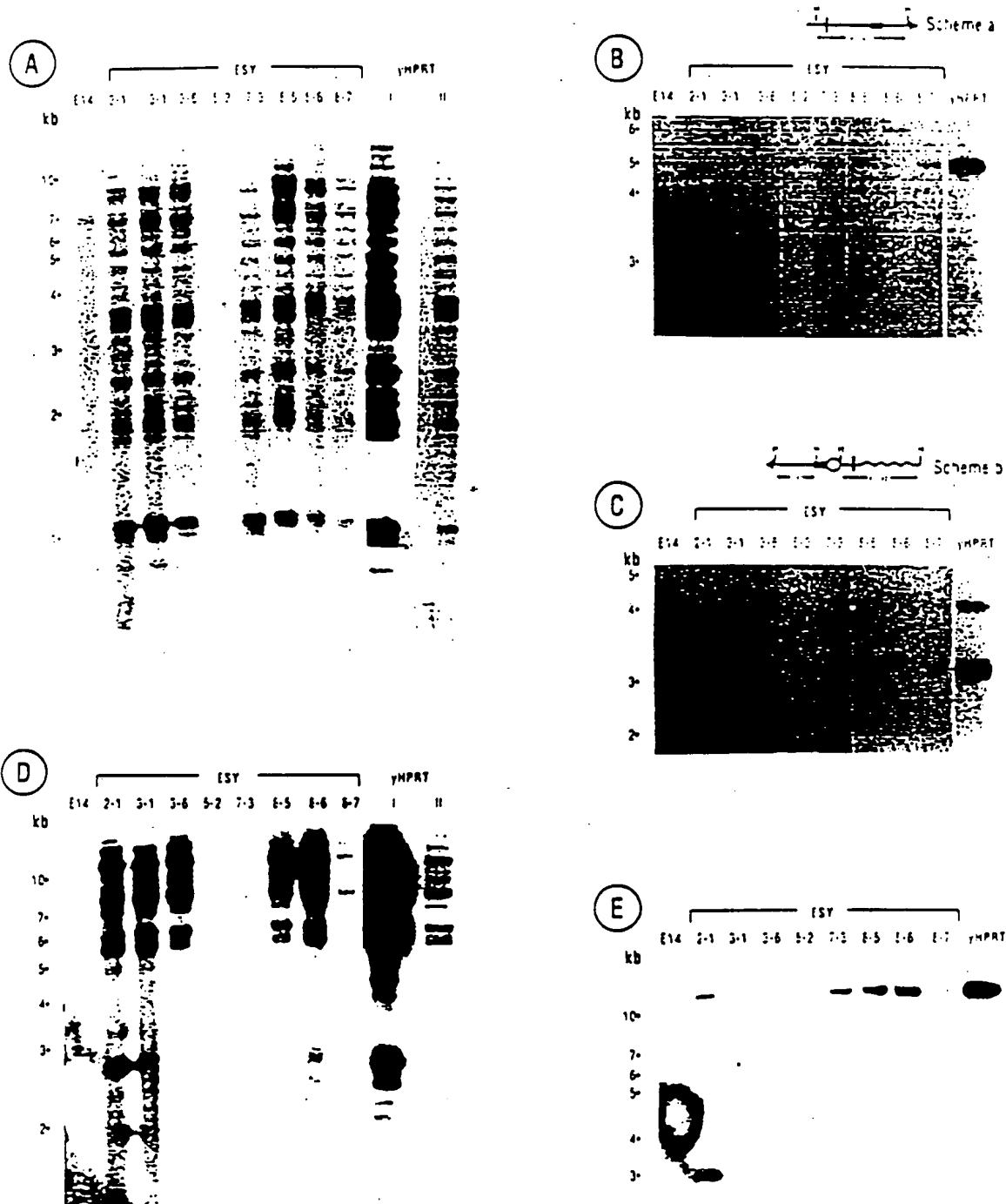


Figure 12

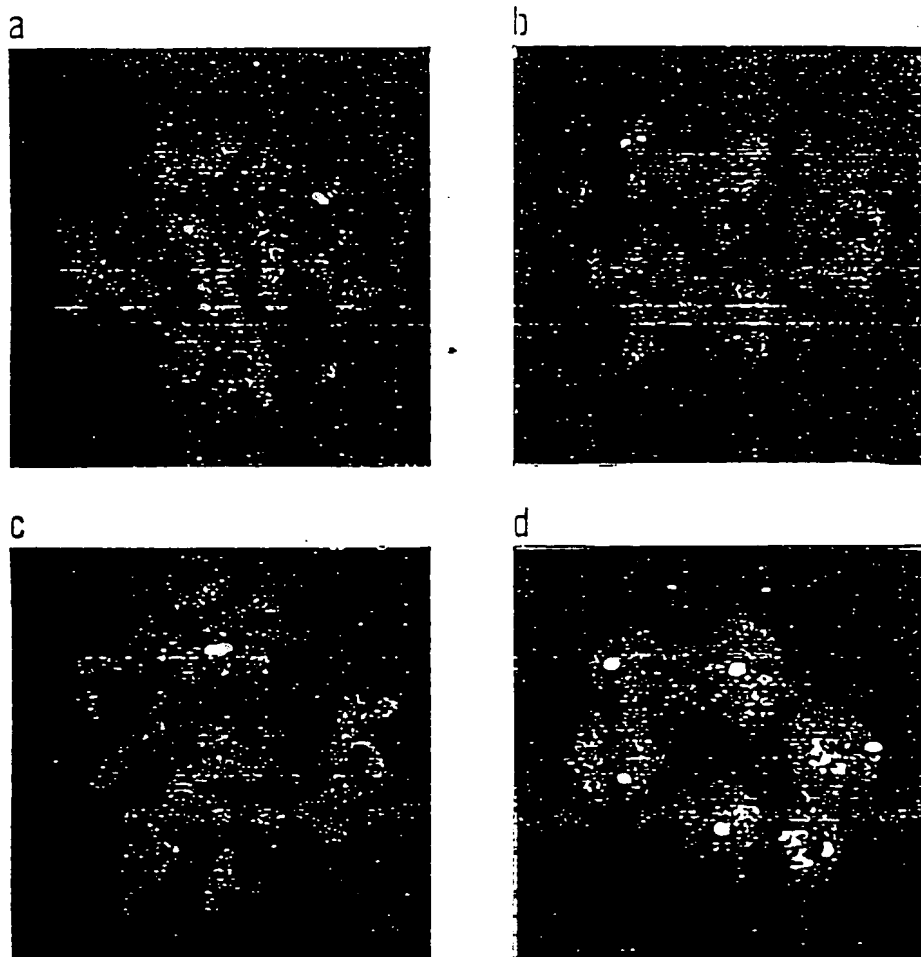


Figure 13

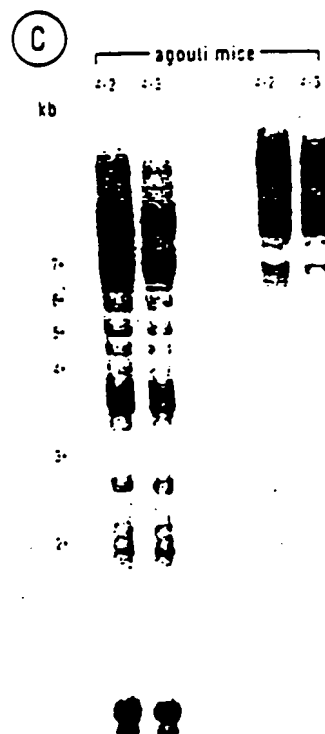
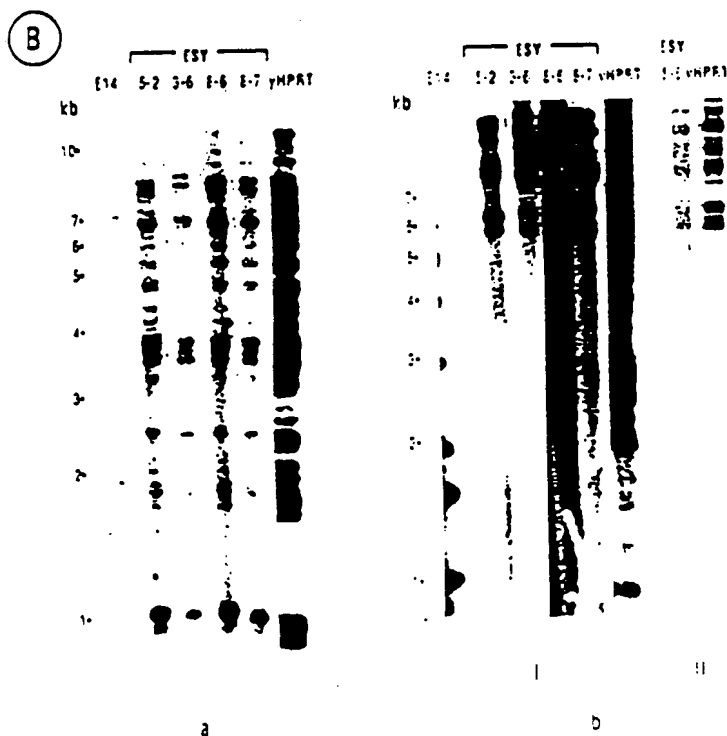
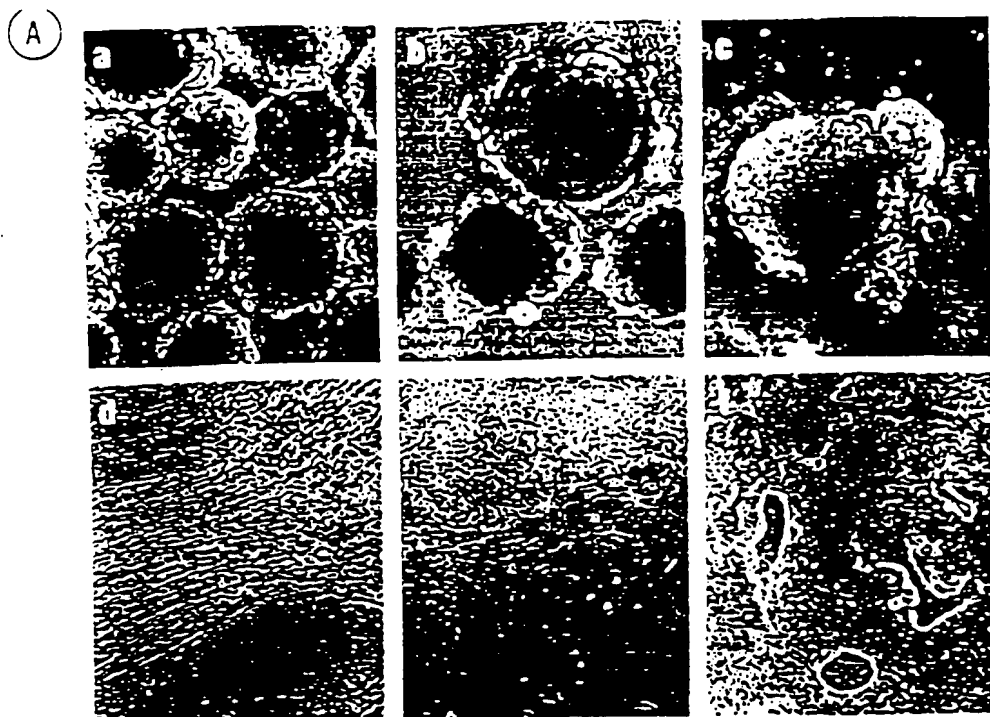


Figure 14

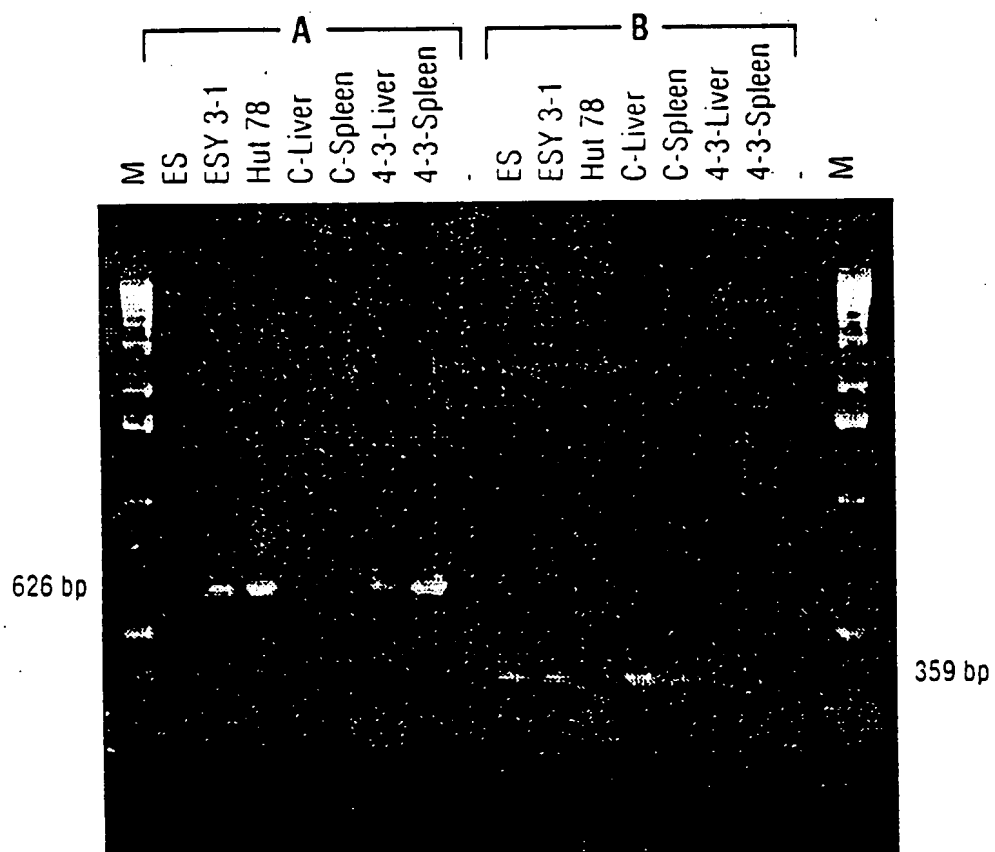
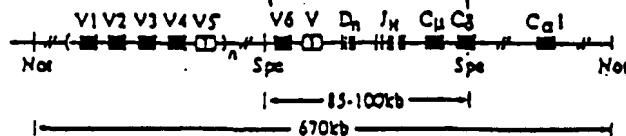


Figure 15

Interspersed members of V1,V2,V3,V4,V5



JS58.V31.VGAM3.B



5' 3'

TEL HIS3 CEN V6V Dn 1H Cα Cβ LEU2 TEL

SpeI SpeI

mouse 558 Neo mouse Cα-Cα

85-100kb

Figure 16

Mouse Breeding Scheme

Cross IA.

heterozygous inactive Murine IgH
X
heterozygous inactive Murine IgK

MIgH (inactive) MIgK
MIgH MIgK
X
MIgH MIgK (inactive)
MIgH MIgK

↓

F1 (cross I A)

MIgH (inactive) MIgK (inactive)
MIgH MIgK

Cross I B.

heterozygous Human IgH
X
heterozygous Human IgK

MIgH MIgK HIgH
MIgH MIgK
X
MIgH MIgK HIgK
MIgH MIgK

↓

F1 (cross I B)

MIgH MIgK HIgH HIgK
MIgH MIgK

Cross II.

F1 (cross I A) x F1 (cross I B)

↓

F2 Quadruple Heterozygotes

MIgH (inactive) MIgK (inactive) HIgH HIgK
MIgH MIgK

Cross III.

Intercross F2 mice

↓

F3 DOUBLE Homozygotes

MIgH (inactive) MIgK (inactive) HIgH HIgK
MIgH (inactive) MIgK (inactive)

Figure 17

MAMMALIAN HOST GENOTYPES

<u>Hetero- or Hemi-zygous Mice</u>	<u>Intercross Product Mice*</u>
I. <u>ΔmIgL</u> <u>mIgH</u> <u>mIgL</u> <u>mIgH</u>	<u>ΔmIgL</u> <u>mIgH</u> <u>ΔmIgL</u> <u>mIgH</u>
II. <u>mIgL</u> <u>ΔmIgH</u> <u>mIgL</u> <u>mIgH</u>	<u>mIgL</u> <u>ΔmIgH</u> <u>mIgL</u> <u>ΔmIgH</u>
III. <u>mIgL</u> <u>mIgH</u> <u>hIgH</u> <u>mIgL</u> <u>mIgH</u>	<u>mIgL</u> <u>mIgH</u> <u>hIgH</u> <u>mIgL</u> <u>mIgH</u> <u>hIgH</u>
IV. <u>mIgL</u> <u>mIgH</u> <u>hIgL</u> <u>mIgL</u> <u>mIgH</u>	<u>mIgL</u> <u>mIgH</u> <u>hIgL</u> <u>mIgL</u> <u>mIgH</u> <u>hIgL</u>
V. Animal I X Animal II	
<u>ΔmIgL</u> <u>mIgH</u> <u>mIgL</u> <u>ΔmIgH</u>	<u>ΔmIgL</u> <u>ΔmIgH</u> <u>ΔmIgL</u> <u>ΔmIgH</u>
VI. Animal III X Animal V	
<u>mIgL</u> <u>mIgH</u> <u>hIgH</u> <u>ΔmIgL</u> <u>ΔmIgH</u>	<u>ΔmIgL</u> <u>ΔmIgH</u> <u>hIgH</u> and <u>ΔmIgL</u> <u>ΔmIgH</u> <u>hIgH</u> <u>ΔmIgL</u> <u>ΔmIgH</u> <u>hIgH</u>
VII. Animal IV X Animal V	
<u>mIgL</u> <u>mIgH</u> <u>hIgL</u> <u>ΔmIgL</u> <u>ΔmIgH</u>	<u>ΔmIgL</u> <u>ΔmIgH</u> <u>hIgL</u> and <u>ΔmIgL</u> <u>ΔmIgH</u> <u>hIgL</u> <u>ΔmIgL</u> <u>ΔmIgH</u> <u>hIgL</u>
VIII. Animal VI X Animal VII	
<u>ΔmIgL</u> <u>ΔmIgH</u> <u>hIgL</u> <u>hIgH</u> <u>ΔmIgL</u> <u>ΔmIgH</u>	<u>ΔmIgL</u> <u>ΔmIgH</u> <u>hIgL</u> <u>hIgH</u> <u>ΔmIgL</u> <u>ΔmIgH</u> <u>hIgL</u> <u>hIgH</u>
<u>mIgL</u> <u>mIgH</u> <u>hIgL</u> <u>hIgH</u> <u>ΔmIgL</u> <u>ΔmIgH</u>	<u>ΔmIgL</u> <u>ΔmIgH</u> <u>hIgL</u> <u>hIgH</u> and <u>ΔmIgL</u> <u>ΔmIgH</u> <u>hIgL</u> <u>hIgH</u> <u>ΔmIgL</u> <u>ΔmIgH</u> <u>hIgH</u>
IX. Animal III X Animal IV	
<u>mIgL</u> <u>mIgH</u> <u>hIgL</u> <u>hIgH</u> <u>mIgL</u> <u>mIgH</u>	<u>mIgL</u> <u>mIgH</u> <u>hIgL</u> <u>hIgH</u> <u>mIgL</u> <u>mIgH</u> <u>hIgL</u> <u>hIgH</u>
X. Animal II X Animal IX	
<u>mIgL</u> <u>ΔmIgH</u> <u>hIgL</u> <u>hIgH</u> <u>mIgL</u> <u>mIgH</u>	<u>mIgL</u> <u>ΔmIgH</u> <u>hIgL</u> <u>hIgH</u> and <u>mIgL</u> <u>ΔmIgH</u> <u>hIgL</u> <u>hIgH</u> <u>mIgL</u> <u>ΔmIgH</u>
XI. Animal I X Animal IX	
<u>ΔmIgL</u> <u>mIgH</u> <u>hIgL</u> <u>hIgH</u> <u>mIgL</u> <u>mIgH</u>	<u>ΔmIgL</u> <u>mIgH</u> <u>hIgL</u> <u>hIgH</u> and <u>ΔmIgL</u> <u>mIgH</u> <u>hIgL</u> <u>hIgH</u> <u>ΔmIgL</u> <u>mIgH</u>

*Not all possible genotypes from intercrosses are shown.

Δ = functionally inactive locus
m = mouse endogenous gene
h = human transgene
IgH = immunoglobulin heavy chain
IgL = immunoglobulin light chain